

AMENDMENTS TO THE CLAIMS:

The following Listing of Claims replaces all prior Listings and versions of claims in the above-identified application.

Listing of Claims

1-39. (Canceled).

40. (Currently Amended) A method to produce glucosamine by fermentation, comprising:

a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a bacterium or yeast microorganism which comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase compared to the unmodified glucosamine-6-phosphate synthase, wherein said genetic modification is selected from the group consisting of:

i) transformation of said microorganism with a recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate synthase, wherein said synthase has glucosamine-6-phosphate synthase activity; and

ii) genetic modification of the coding region of a gene encoding a bacterial or yeast glucosamine-6-phosphate synthase, wherein said genetic modification results in at least one nucleic acid modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said coding region of said gene, wherein said at least one nucleotide modification results in increased glucosamine-6-phosphate synthase activity compared to the unmodified glucosamine-6-phosphate synthase;

wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said bacterium or yeast microorganism; and

- b) recovering and purifying said product.

41. (Currently Amended) The method of Claim 40, wherein said glucosamine-6-phosphate is intracellular and said glucosamine is extracellular, wherein said step of recovering comprises a recovering step selected from the group consisting of recovering said glucosamine-6-phosphate from said bacterium or yeast microorganism, recovering said glucosamine from said fermentation medium, and a combination thereof.

42. (Currently Amended) The method of Claim 40, wherein said product is intracellular glucosamine-6-phosphate and said step of recovering comprises isolating said glucosamine-6-phosphate from said bacterium or yeast microorganism.

43. (Previously Presented) The method of Claim 40, wherein said product is intracellular glucosamine-6-phosphate and said step of recovering further comprises dephosphorylating said glucosamine-6-phosphate to produce glucosamine.

44. (Previously Presented) The method of Claim 40, wherein said step of culturing comprises maintaining said source of carbon at a concentration of from about 0.5% to about 5% in said fermentation medium.

45. (Previously Presented) The method of Claim 40, wherein said step of culturing is performed at a temperature from about 30°C to about 40°C.

46. (Previously Presented) The method of Claim 40, wherein said step of culturing is performed at about 30°C.

47. (Previously Presented) The method of Claim 40, wherein said step of culturing is performed in a fermentor.

48. (Previously Presented) The method of Claim 47, wherein said step of

culturing is performed under conditions wherein glucose is added to said fermentation medium at a rate in which glucose accumulation in said fermentation medium is undetectable.

49. (Previously Presented) The method of Claim 47, wherein said step of culturing is performed so that an excess of glucose is maintained.

50. (Previously Presented) The method of Claim 40, wherein said step of culturing produces and accumulates at least about 21 mg/L of said product.

51. (Previously Presented) The method of Claim 40, wherein said step of culturing produces and accumulates at least about 1 g/L of said product.

52. (Previously Presented) The method of Claim 40, wherein said step of culturing produces and accumulates at least about 5 g/L of said product.

53. (Currently Amended) The method of Claim 40, wherein said genetic modification comprises transformation of said bacterium or yeast microorganism with a recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate synthase ~~that~~ which has glucosamine-6-phosphate synthase enzymatic activity, wherein said recombinant nucleic acid molecule is operatively linked to a transcription control sequence.

54. (Currently Amended) The method of Claim 53, wherein said recombinant nucleic acid molecule is integrated into the genome of said bacterium or yeast microorganism.

55. (Currently Amended) The method of Claim 53, wherein said recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate synthase comprises a genetic modification which increases the activity of said glucosamine-6-phosphate synthase compared to the unmodified glucosamine-6-phosphate synthase.

56. (Currently Amended) The method of Claim 40, wherein said recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate synthase or said gene encoding said a bacterial or yeast glucosamine-6-phosphate synthase comprises a genetic modification which reduces glucosamine-6-phosphate product inhibition of said glucosamine-6-phosphate synthase compared to the unmodified glucosamine-6-phosphate synthase.

57. (Currently Amended) The method of Claim 40, wherein said bacterium or yeast microorganism has at least one additional genetic modification in a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II^{Nag}, phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridylyltransferase, phosphofructokinase, Enzyme II^{Glc} of the PEP:glucose PTS, and EIIM,P/III^{Man} of the PEP:mannose PTS, wherein said genetic modification decreases the activity of said protein compared to the unmodified protein.

58. (Currently Amended) The method of Claim 40, wherein said bacterium or yeast microorganism has at least one additional genetic modification in a gene encoding a phosphatase, wherein said genetic modification increases the activity of said phosphatase compared to the unmodified phosphatase.

59. (Currently Amended) The method of Claim 40, wherein said bacterium or yeast microorganism has additional modifications in genes encoding the following proteins: *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase and *N*-acetyl-glucosamine-specific enzyme II^{Nag};

wherein said genetic modification decreases the activity of said proteins compared to the unmodified proteins.

60. (Currently Amended) The method of Claim 40, wherein said bacterium or yeast microorganism has additional modifications in genes encoding *N*-acetylglucosamine-6-phosphate deacetylase and glucosamine-6-phosphate deaminase; wherein said genetic modification decreases the activity of said proteins compared to the unmodified proteins.

61. (Previously Presented) The method of Claim 60, wherein said genetic modification is a deletion of at least a portion of said genes.

62. (Currently Amended) The method of Claim 40, wherein said bacterium or yeast microorganism is a bacterium ~~selected from the group consisting of bacteria and yeast~~.

63. (Currently Amended) The method of Claim 62 ~~[[40]]~~, wherein said bacterium microorganism is a bacterium of the genus *Escherichia*.

64. (Currently Amended) The method of Claim 63 ~~[[40]]~~, wherein said bacterium microorganism is *Escherichia coli*.

65. (Currently Amended) The method of Claim 64, wherein said bacterium microorganism comprises at least one additional genetic modification which is a mutation in an *Escherichia coli* gene selected from the group consisting of *nagA*, *nagB*, *nagC*, *nagD*, *nagE*, *manXYZ*, *glmM*, *pfkB*, *pfkA*, *glmU*, *glmS*, *ptsG* and a phosphatase gene, wherein said genetic modification decreases the activity of a protein encoded by said gene compared to the unmodified protein.

66. (Currently Amended) The method of Claim 40, wherein said bacterium or yeast microorganism is a yeast.

67. (Currently Amended) A recombinant bacterium or yeast microorganism for

producing glucosamine by a biosynthetic process, said bacterium or yeast microorganism being transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast glucosamine-6-phosphate synthase, said nucleic acid sequence being operatively linked to a transcription control sequence and comprising, in the coding region of the nucleic acid sequence, a genetic modification which increases the activity of said glucosamine-6-phosphate synthase compared to the unmodified glucosamine-6-phosphate synthase;

wherein expression of said nucleic acid sequence increases production of glucosamine by said bacterium or yeast microorganism.

68. (Currently Amended) The recombinant bacterium or yeast microorganism of Claim 67, wherein said bacterium or yeast microorganism has at least one additional genetic modification in a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II^{Neq}, phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridylyltransferase, phosphofructokinase, Enzyme II^{Glc} of the PEP:glucose PTS, and EIIM,P/III^{Man} of the PEP:mannose PTS, wherein said genetic modification decreases the activity of said protein compared to the unmodified protein.

69. (Currently Amended) The recombinant bacterium or yeast microorganism of Claim 67, wherein said bacterium or yeast microorganism has at least one additional genetic modification in a gene encoding a phosphatase, wherein said genetic modification increases the activity of said phosphatase compared to the unmodified phosphatase.

70. (Currently Amended) A method to produce glucosamine by fermentation, comprising:

- a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a bacterium or yeast microorganism having

at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase compared to the unmodified glucosamine-6-phosphate synthase, wherein said genetic modification is selected from the group consisting of:

- i) transformation of said bacterium or yeast microorganism with a recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate synthase which has glucosamine-6-phosphate synthase activity; and
- ii) genetic modification of a gene encoding a bacterial or yeast glucosamine-6-phosphate synthase that increases the activity of said glucosamine-6-phosphate synthase compared to the unmodified glucosamine-6-phosphate synthase, and wherein said genetically modified bacterium or yeast is produced by a process comprising the steps of:
 - (1) generating modifications in an isolated nucleic acid molecule comprising a nucleic acid sequence encoding a bacterial or yeast glucosamine-6-phosphate synthase to create a plurality of modified nucleic acid sequences;
 - (2) transforming bacteria or yeast microorganisms with said modified nucleic acid sequences to produce genetically modified bacteria or yeast microorganisms;
 - (3) screening said genetically modified bacteria or yeast microorganisms for glucosamine-6-phosphate synthase activity; and,
 - (4) selecting said genetically modified bacteria or yeast microorganisms which have increased glucosamine-6-phosphate synthase activity compared to the unmodified glucosamine-6-phosphate synthase;

wherein said step of culturing produces a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and,

- b) recovering said product.

71. (Currently Amended) A method to produce glucosamine by fermentation, comprising:

- a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a bacterium or yeast microorganism that expresses ~~has been transformed with~~ a recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate synthase, wherein said synthase has glucosamine-6-phosphate synthase enzymatic activity, and wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said bacterium or yeast microorganism; and
- b) recovering and purifying said product.

72. (Currently Amended) The method of Claim 71, wherein said glucosamine-6-phosphate synthase comprises a genetic modification which reduces glucosamine-6-phosphate product inhibition of said glucosamine-6-phosphate synthase compared to the unmodified glucosamine-6-phosphate synthase.

73. (Canceled)

74. (Currently Amended) The method of Claim 40, wherein the glucosamine-6-phosphate synthase is a yeast glucosamine-6-phosphate synthase ~~from a~~ microorganism.

75. (Currently Amended) The method of Claim 40, wherein the glucosamine-6-phosphate synthase is a bacterial glucosamine-6-phosphate synthase ~~from a~~ bacterium.

76. (Previously Presented) The method of Claim 40, wherein said product is extracellular glucosamine-6-phosphate and said step of recovering comprises recovering said glucosamine-6-phosphate from said fermentation medium.

77. (New) The method of Claim 75, wherein the bacterial glucosamine-6-phosphate synthase is from a bacterium of the genus *Escherichia*.

78. (New) The method of Claim 71, wherein said glucosamine-6-phosphate synthase is a bacterial glucosamine-6-phosphate synthase.

79. (New) The method of Claim 78, wherein said bacterial glucosamine-6-phosphate synthase is from a bacterium of the genus *Escherichia*.

80. (New) The method of Claim 71, wherein the bacterium or yeast further comprises a partial or complete deletion of a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II^{Nag}, phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridylyltransferase, phosphofructokinase, Enzyme II^{Glc} of the PEP:glucose PTS, and EIIM,P/III^{Man} of the PEP:mannose PTS, wherein said partial or complete deletion decreases the activity of said protein compared to the unmodified protein.

81. (New) The method of Claim 80, wherein the bacterium or yeast is a bacterium.

82. (New) A method to produce glucosamine by fermentation, comprising:
a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a bacterium or yeast that expresses a recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-

phosphate synthase, wherein said synthase has glucosamine-6-phosphate synthase enzymatic activity and comprises a genetic modification that reduces the glucosamine-6-phosphate product inhibition of said glucosamine-6-phosphate synthase compared to the unmodified glucosamine-6-phosphate synthase, and wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said bacterium or yeast; and

b) recovering and purifying said product.

83. (New) The method of Claim 82, wherein the bacterium or yeast is a bacterium.

84. (New) The method of Claim 83, wherein the bacterial or yeast glucosamine-6-phosphate synthase is a bacterial glucosamine-6-phosphate synthase.

85. (New) The method of Claim 82, wherein the bacterium or yeast is a yeast.

86. (New) The method of Claim 85, wherein the bacterial or yeast glucosamine-6-phosphate synthase is a yeast glucosamine-6-phosphate synthase.

87. (New) A method to produce glucosamine by fermentation, comprising:

a) culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a bacterium or yeast that expresses a recombinant nucleic acid molecule encoding a bacterial or yeast glucosamine-6-phosphate synthase, wherein said synthase has glucosamine-6-phosphate synthase enzymatic activity, and wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said bacterium or yeast; and

b) recovering and purifying said product.

88. (New) The method of Claim 87, wherein the bacterium or yeast is a bacterium.

89. (New) The method of Claim 88, wherein the bacterial or yeast glucosamine-6-phosphate synthase is a bacterial glucosamine-6-phosphate synthase.

90. (New) The method of Claim 87, wherein the bacterium or yeast is a yeast.

91. (New) The method of Claim 90, wherein the bacterial or yeast glucosamine-6-phosphate synthase is a yeast glucosamine-6-phosphate synthase.